

1 **Supplemental data**

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3 **Supplementary Figure 1: Experimental model and procedure schedule.**

4 All mice were randomly divided into four groups (n = 8 per group): Group A (Control, Con): Mice  
5 received saline treatment and sham EA (Sham-EA; acupuncture without electrical stimulation).

6 Group B: Mice with DSS-induced colitis received sham EA. Group C (Low-intensity EA, EAL):

7 Mice with DSS-induced colitis received EA at 0.5 mA, 10 Hz for 15 min. Group D (High-intensity

8 EA, EAH): Mice with DSS-induced colitis received EA at 1 mA, 10 Hz for 15 min. (B) ST36

9 (Zusanli) is located on the posterolateral side of the knee, approximately 2 mm below the fibular

10 head. (C) Mice were immobilized in a tube, and EA was applied to both hind limbs. (D) EA was

11 performed with three mice simultaneously.

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14 **Supplementary Figure 2: Electroacupuncture (EA) at ST36 exerts no significant regulatory**  
15 **effect on the number of immune cells in DSS-induced mice.**

16 (A–G) Representative flow cytometric analyses of immune cells from colonic tissues. The

17 percentages of total immune cells are shown (n = 3 per group). Immune cell subsets were

18 identified and characterized based on the following phenotypic markers:

19 Eosinophils: Live CD45<sup>+</sup>CD11b<sup>+</sup>SiglecF<sup>+</sup>CD11c<sup>-</sup>;

20 Dendritic cells (DCs): Live CD45<sup>+</sup>CD11b<sup>+</sup>SiglecF<sup>-</sup>CD11c<sup>+</sup>MHC-II<sup>+</sup>F4/80<sup>-</sup>;

21 Neutrophils: Live CD45<sup>+</sup>CD11b<sup>+</sup>SiglecF<sup>-</sup>CD11c<sup>-</sup>Ly6G<sup>+</sup>Ly6C<sup>high</sup>;

22 Ly6C<sup>high</sup> monocytes: Live CD45<sup>+</sup>CD11b<sup>+</sup>SiglecF<sup>-</sup>CD11c<sup>-</sup>Ly6C<sup>high</sup>;

23 Ly6C<sup>low</sup> monocytes: Live CD45<sup>+</sup>CD11b<sup>+</sup>SiglecF<sup>-</sup>CD11c<sup>-</sup>Ly6C<sup>low</sup>;

24 Macrophages: Live CD45<sup>+</sup>CD11b<sup>+</sup>SiglecF<sup>-</sup>CD11c<sup>-</sup>Ly6C<sup>-</sup>Ly6G<sup>-</sup>MHC-II<sup>+</sup>F4/80<sup>+</sup>;

25 CD8<sup>+</sup> T cells: Live CD45<sup>+</sup>CD8<sup>+</sup>;

26 B cells: Live CD45<sup>+</sup>B220<sup>+</sup>;

27 CD4<sup>+</sup> T cells: Live CD45<sup>+</sup>CD4<sup>+</sup>CD11b<sup>-</sup>B220<sup>-</sup>CD8<sup>-</sup>.

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29 **Supplementary Figure 3: Electroacupuncture (EA) at ST36 (Zusanli) exerts a protective**  
30 **effect in dextran sulfate sodium (DSS)-induced colitis by regulating the density of**  
31 **TH-positive neurons in the colon.**

32 (A) Colonic tissues from six mice per group were harvested and processed for whole-tissue TH

33 immunolabeling. Representative 3D-projection images (cross-sectional view) of 2-mm thick tissue

34 sections acquired via light sheet imaging at 1.26× magnification are shown.  
 35 (B) TH-positive catecholaminergic axons (TH<sup>+</sup>) and VAcHT-positive cholinergic axons (SNAP25<sup>+</sup>)  
 36 in the colon were quantified. Scale bars = 300 μm, n = 3, mean ± SEM, \*P < 0.01 (Student's  
 37 t-test).

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39 **Supplementary Figure4: Three-dimensional (3D) animation of neurons in the enteric**  
 40 **nervous system from colonic tissues.**

41 Whole-tissue immunolabeling reveals the presence of SNAP25<sup>+</sup> and TH<sup>+</sup> neurons in the enteric  
 42 nervous system.

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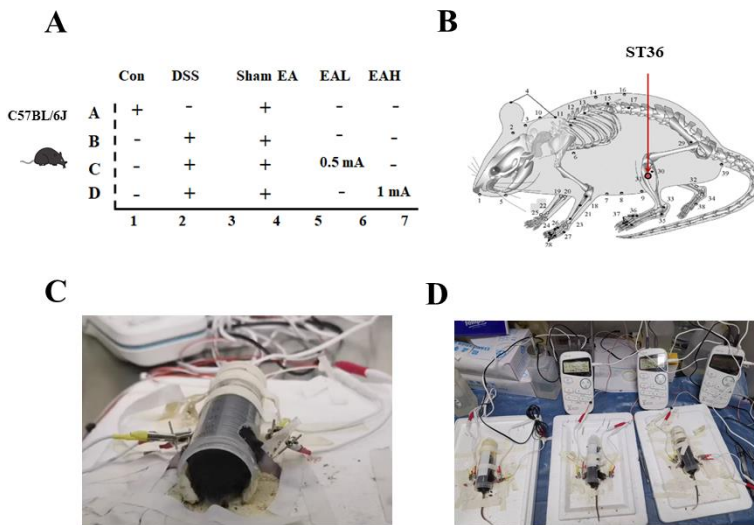
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46 **Supplementary Figures**

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S.Fig.1



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49 **Supplementary Figure 1: Experimental model and procedure schedule.**

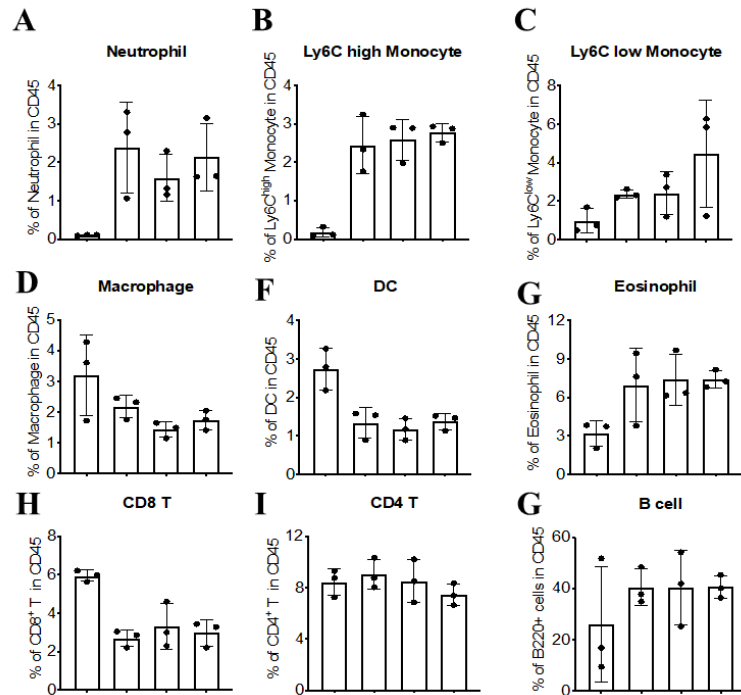
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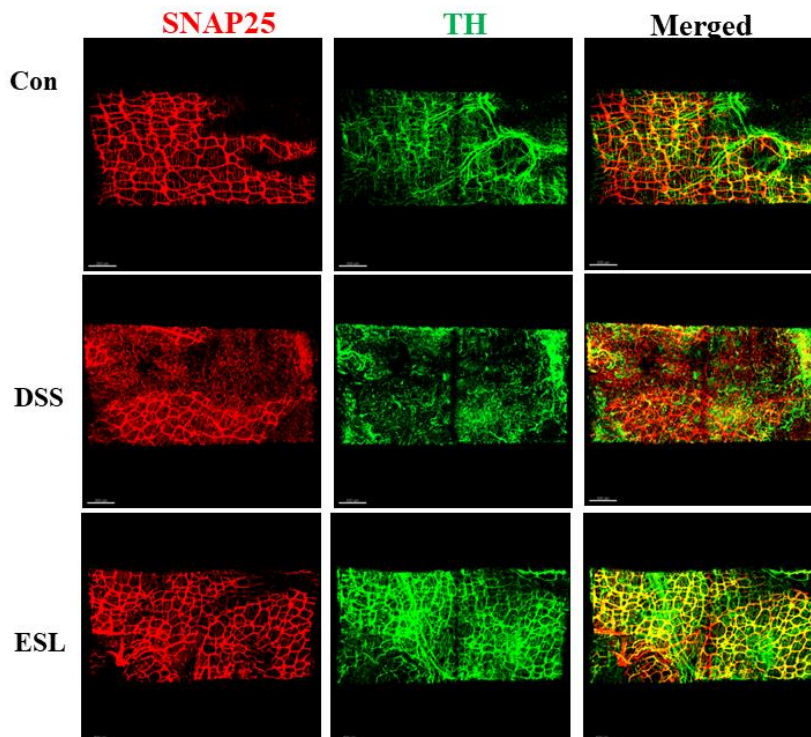
S.Fig.2



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55 **Supplementary Figure 2:** Electroacupuncture (EA) at ST36 exerts no significant regulatory  
56 effect on the number of immune cells in DSS-induced mice.

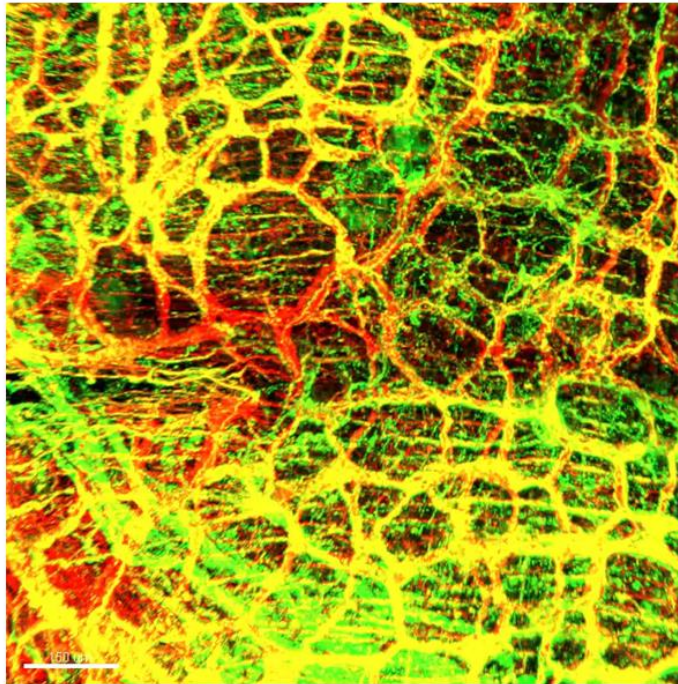
S. Fig.3



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58 **Supplementary Figure 3:** Electroacupuncture (EA) at ST36 (Zusanli) exerts a protective effect in  
59 dextran sulfate sodium (DSS)-induced colitis by regulating the density of TH-positive neurons in  
60 the colon.

**S. Fig.4**



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62 **Supplementary Figure 4:** Three-dimensional (3D) animation of neurons in the enteric nervous  
63 system from colonic tissues.

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85 Supplementary Table.1

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Reagent or Resource	Source	Identifier
<b>Antibodies</b>		
anti-tyrosine hydroxylase antibody	Sigma-Aldrich	AB152
purified anti-SNAP-25 antibody	BioLegend	836304
Goat Anti-Mouse IgG H&L (594)	Abcam	ab150116
Goat Anti-Mouse IgG H&L (647)	Abcam	ab150087
anti-mouse CD45.2 (AF700)	Thermo Fisher Scientific	56-0454-82
anti-mouse TCR $\beta$ (BV605)	BioLegend	109241
anti-mouse CD4 (PE)	BioLegend	100511
anti-mouse CD8 $\alpha$ (AF647)	BioLegend	100727
Anti-mouse Ly6G(APC)	BioLegend	127613
Anti-mouse SiglecF(APC/CY7)	BioLegend	155531
Anti-mouse B220+(APC/CY7)	BioLegend	103223
Fixable Viability Stain (AF700)	BD Biosciences	564997
<b>Chemicals</b>		
DSS, Dextran Sodium Sulfate	MP Biomedical	02160110-CF
M.O.M. Blocking Reagent	Vector laboratories	MKB-2213
ProClean 300	Beyotime	ST853-250
Fluo Tissue prepared slide kit	Rapiclear	RC152001
Heparin	MCE	HY-17567
Normal donkey serum	jackson	017-000-121
Normal Goat Serum	jackson	005-000-121
Hematoxylin and Eosin Staining Kit	Beyotime	C0105M

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